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INTERACTION OF ACID SULFATES AND THE RESPIRATORY SYSTEM

by

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Grant No. RO-80378

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16. ABSTRACT The extent of neutralization of inhaled H_2SO_4 aerosol by endogenous NH_3 has been measured in the surgically isolated upper airways of anesthetized dogs. Neutralization was observed to be inversely proportional to particle size. At a given particle size neutralization is related to both the route of entry and the flow rate. It is greater for entry via the mouth than the nose and greater for a flow of 0.1 1/sec than 0.2 1/sec.

Characterization of the respiratory response of the guinea pig exposed to an aerosol of histamine dihydrochloride is described as a positive control for future animal exposure studies. Dynamic lung compliance exhibited a progressive decrease during a ten-minute exposure to an aerosol mass concentration of 885 ug/m^3; guinea pigs which responded to a lower concentration of 565 ug/m^3 exhibited the same progressive decrease in compliance.

The potential for ammonia produced naturally in the mouth to modify the effects of inhaled sulfuric acid aerosol particles was investigated in healthy adults. For acid particles with a mass median diameter of 0.65 um, at a concentration of 500 ug/m^3, neutralization should have been nearly complete during quiet mouth breathing. This situation was compared to moderate exercise where neutralization should be greatly reduced. Using standard measurements of lung mechanical function and a measurement of density dependence of expiratory flow, there was evidence for mild constriction of small airways when exposure occurred during intermittent exercise. The effect of acid aerosol, whether neutralized or not, was minor, however, compared to the independent influence of exercise in briefly constricting large airways.

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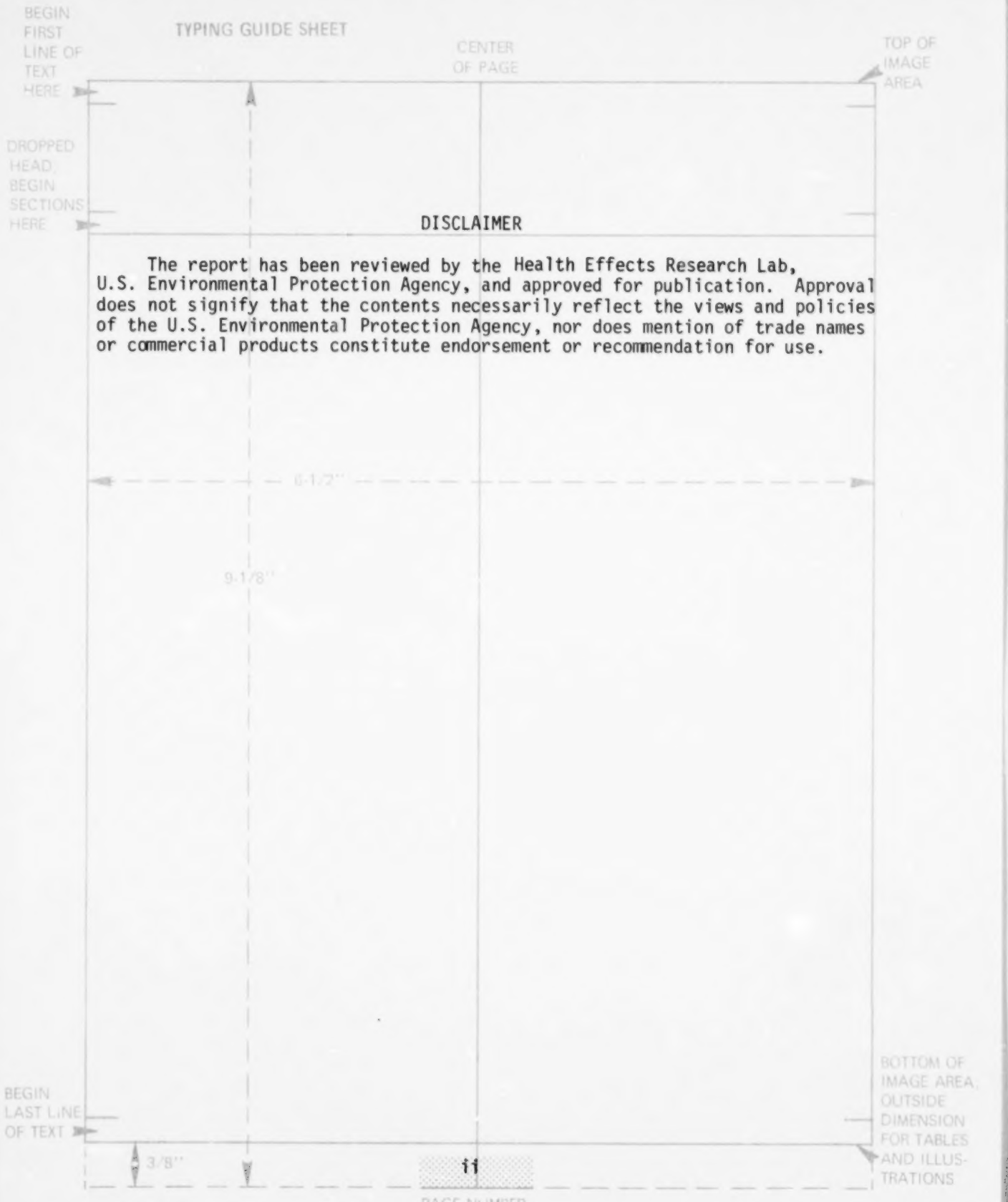
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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The objective of the research performed under this grant was to study certain aspects of the interaction of acid sulfate aerosol particles with the respiratory system.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

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ABSTRACT

MEASUREMENTS OF RESPIRATORY AMMONIA AND THE CHEMICAL NEUTRALIZATION OF INHALED SULFURIC ACID AEROSOL IN ANESTHETIZED DOGS

The extent of neutralization of inhaled H_2SO_4 aerosol by endogenous NH_3 has been measured in the surgically isolated upper airways of anesthetized dogs. Neutralization was observed to be inversely proportional to particle size. H_2SO_4 particles with initial dry diameters of 0.5 um and 1.0 um underwent 0.28 (± 0.08) and 0.06 (± 0.06)% neutralization per ppb of laryngeal NH_3 respectively during passage through the mouth and out of the larynx at a flow of 0.1 l/sec. At a given particle size neutralization is related to both the route of entry and the flow rate. It is greater for entry via the mouth than the nose and greater for a flow of 0.1 l/sec than 0.2 l/sec. These results are consistent with a reaction that is limited by the rate of NH_3 diffusion to the particle's surface.

RESPIRATORY RESPONSE OF GUINEA PIGS TO AEROSOLS
I HISTAMINE AEROSOL CONTROL STUDY

Characterization of the respiratory response of the guinea pig exposed to an aerosol of histamine dihydrochloride is described as a positive control for future animal exposure studies. Dynamic lung compliance exhibited a progressive decrease during a ten-minute exposure to an aerosol mass concentration of 885 ug/m^3; guinea pigs which responded to a lower concentration of 565 ug/m^3 exhibited the same progressive decrease in compliance. This observation is consistent with the hypothesis that a fraction of the guinea pig population is hypersensitive. Comparisons are made with findings of other investigators. These results will be used as an index of sensitivity of our biological preparation in the analysis of our data on pulmonary functional response of guinea pigs to sulfate compounds in atmospheric aerosols.

THE INFLUENCE OF EXERCISE AND ENDOGENOUS AMMONIA ON PULMONARY IRRITATION BY SULFURIC ACID AEROSOL

The potential for ammonia produced naturally in the mouth to modify the effects of inhaled sulfuric acid aerosol particles was investigated in healthy adults. If significant chemical neutralization were to occur before deposition the irritant potency of the aerosol might be significantly reduced. For acid particles with a mass median diameter of 0.65 um, at a concentration of 500 ug/m^3, neutralization should have been

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nearly complete during quiet mouth breathing. This situation was compared to moderate exercise where neutralization should be greatly reduced. Using standard measurements of lung mechanical function and a measure of density dependence of expiratory flow, there was evidence for mild constriction of small airways when exposure occurred during intermittent exercise. The effect of acid aerosol, whether neutralized or not, was minor, however, compared to the independent influence of exercise in briefly constricting large airways.

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SECTION 1

INTRODUCTION

The objective of the research performed under this grant was to study certain aspects of the interaction of acid sulfate aerosol particles with the respiratory system.

Three specific areas of study were undertaken and completed.

1. To assess the importance of respiratory NH_3 in neutralizing inhaled acid sulfate aerosols (H_2SO_4) and in altering the irritant effects of these aerosols.

2. To study the respiratory response of guinea pigs to two size-distributions of H_2SO_4 aerosols encountered in community air (nuclei and accumulation modes).

3. To study the effects of H_2SO_4 aerosols (accumulation mode) on pulmonary function in healthy young adults during rest and exercise.

The physical and chemical aspects of objective 1 are presented in Section 2 of the report. The extrapolation and application of the principles developed there are applied to human exposure in Sections 2 and 4. The framework for the analysis of data obtained under objective 2 is presented in Section 3. There is a large body of data pertaining to objective 2 that has been reduced but not yet analyzed. Analysis required the completion of the guinea pig histamine exposure and data analysis (reported here) as a reference and quality assurance baseline. The analysis will be completed in early 1981.

The sections of this final report are presented as manuscripts for publication in the open literature. Following are the submission dates and locations.

1. T.V. Larson, N.R. Frank, D.S. Covert, D. Holub and M.S. Morgan, Measurement of Respiratory Ammonia and the Chemical Neutralization of Inhaled Sulfuric Acid Aerosol in Anestetized Dogs, American Review of Respiratory Disease.

2. N.R. Horike, M.S. Morgan, N.R. Frank, The Respiratory Reponse of Guinea Pigs to Aerosols: I Histamine Aerosol Control Study, Environmental Research.

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3. M.S. Morgan, D.S. Covert, T.V. Larson, N.R. Frank, The Influence of Exercise and Endogenous Ammonia on Pulmonary Irritation by Sulfuric Acid Aerosol, Environmental Research.

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SECTION 2

MEASUREMENTS OF RESPIRATORY AMMONIA AND THE CHEMICAL NEUTRALIZATION OF INHALED SULFURIC ACID AEROSOL IN ANESTHETIZED DOGS

Sulfuric acid (H_2SO_4) and its neutralization products [NH_4HSO_4, $(NH_4)_2SO_4$] with ammonia (NH_3) are important components of the submicrometer-diameter particles suspended in the ambient atmosphere (1, 2, 3). Inhalation of H_2SO_4 aerosol at concentrations 1 mg/m^3 for one hour has been shown to affect mucocilliary clearance rates in humans, whereas inhalation of $(NH_4)_2SO_4$ aerosol at the same concentration has no effect (4). We have hypothesized that during inhalation acid particles undergo neutralization by respiratory NH_3 as they course the upper airways (5, 6). To the extent that it is the acidity of these particles that is irritating, acid neutralization by endogenous NH_3 during inhalation is important.

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2.1 Experimental Procedure

We examined the relationship between respiratory NH_3 and the chemical neutralization of inhaled H_2SO_4 aerosol in surgically isolated upper airways of anesthetized dogs. A total of nine dogs were studied (seven male); their average body weight was 24 kg (range 15 - 35 kg). The dogs were anesthetized with nembutal (25 mg/kg i.v. initial dose; 25 mg, i.v. hourly maintenance dose added to a continuous D/S infusion. Initial NH_3 measurements were made on air sampled from either the nose or mouth during quiet breathing. The NH_3 concentration was determined by collecting the gas in an acid solution and measuring the dissolved NH_4^+ concentration with a gas-sensing electrode. The upper airway was then isolated by severing the trachea at a point four tracheal rings below the larynx. Following the tracheostomy, an NH_3 measurement was made on expired air sampled from the trachea during quiet breathing. At this time a blood sample was taken from the femoral artery for analysis of pH and NH_4^+ concentration. The dog was then paralyzed with flaxedil (40 mg, i.v. initial dose; 20 mg, i.v. 30 min. maintenance dose) and placed on a respiratory pump (v_T = 10 ml/kg body weight; frequency range 20 - 25 min.$^{-1}$). The rectal temperature and CO_2 levels in air expired from the lungs were monitored continuously.

The isolated upper airway of the dog was then connected to the aerosol generation and sampling apparatus as depicted schematically in Figure 2.1. With the nose (or mouth) blocked, vacuum was applied at the larynx and measurements were made on air drawn through the mouth (or nose) and out the larynx at a flow rate of 0.1 l/sec. This flow rate was monitored continuously with a rotameter downstream of the larynx. Measurement of the

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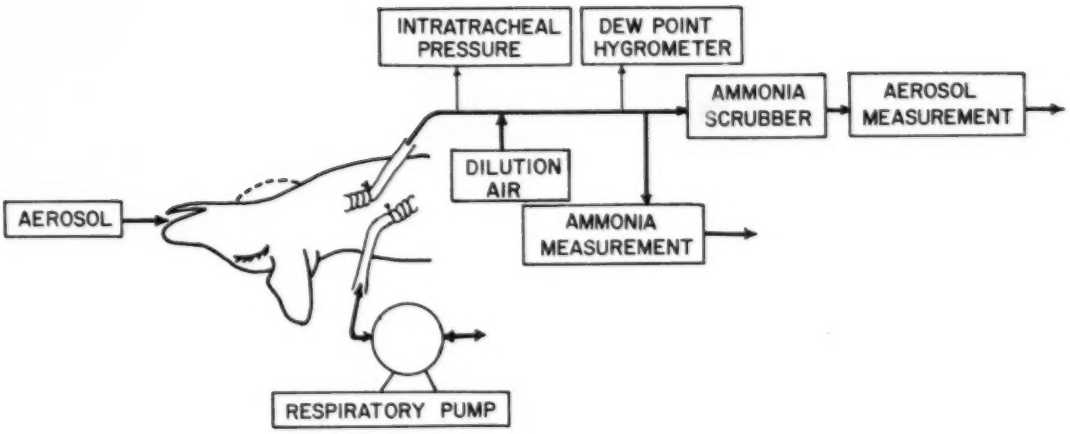
Figure 2.1. Schematic of measurement apparatus.



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pressure drop across the larynx indicated that it was necessary to lift the skin at the throat and to keep the larynx extended under tension in order to reduce the flow restriction to acceptable levels. The nose was blocked by inserting Foley catheters in both nostrils. The mouth was sealed by wrapping it with bandages after placing wetted cotton gauze between the teeth and lips.

The relative humidity (RH) of the air at the larynx was determined by measuring the dry bulb temperature with a thermistor suspended in the air flow and the dew point temperature with a cooled mirror device. A flow restricting needle (21 gauge) kept both the dew point sampling line and sensor at 1/3 atmosphere, to avoid loss of water vapor by condensation. The average time a parcel of air spent traversing the airway between the nose and larynx was measured in five dogs with a gas tracer technique. The technique involved the use of a CO_2 tracer gas, a rapidly responding CO_2 detector (95% rise time = 0.1 sec), and a storage oscilloscope. We measured the concentration of CO_2 tracer gas as it left the larynx and determined the time required to reach 95% of its initial value of 5% (by volume) at the airway inlet.

The dog was then exposed to either 60 ug/m^3 or 100 ug/m^3 of a monodisperse H_2SO_4 aerosol ($\sigma_g < 1.2$) at one of two different particle diameters, either 0.5 um or 1.0 um. This is three to five times the average H_2SO_4 particle mass concentration found in the eastern U.S. during pollution episodes, and respresents the largest of the particles within the "accumulation mode" of the atmospheric aerosol (0.1 um to $\sim$ 1 um in diameter) (8, 9). The particles were produced with a vibrating orifice monodisperse aerosol generator operated at a frequency of $\sim$ 130 kHz. Gaseous NH_3 was removed from the aerosol dilution air by passage through glass-fiber filters pretreated with H_2SO_4. The size distribution of the aerosol was continuously monitored with an optical particle counter. The resolution of particle diameter was limited by the electronic signal processing (pulse height analyzer) to about $\sim$ 10%. This uncertainty is greater than any differences between the measured diameter and the theoretical one calculated from operating parameters of the aerosol generator. Aerosol mass concentration was measured at the point of exposure by weighing samples taken on nuclepore filters. For the higher concentration exposures, the aerosol mass measurements were compared to theoretical values computed from optical measurements of size and number concentration. The mean gravimetric value ($\pm$ standard error) for seven measurements at the point of exposure was 106 ± 7 ug/m^3 compared to a mean optical value of 105 ± 4 ug/m^3. The aerosol was then drawn through the dog's airways and neutralization values were measured at the larynx.

Particles leaving the larynx are surrounded by gaseous NH_3. It is necessary to dilute this aerosol immediately 2.5 to 1 with dry air (RH 20%) and draw it through a denuder, a device that removes the NH_3 from the aerosol but allows the particles to pass through with only negligible losses. The 2.5:1 dilution reduces the RH of the aerosol to 90% at 20°C which slows the reaction rate of NH_3 with acid particles and prevents NH_3 loss to water condensation on the walls of the denuder. The denuder consists of a bundle of about 1900 glass capillary tubes (1.3 mm I.D., 13.4

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cm long) tightly packed inside a larger diameter tube (7.6 cm ID, 18 cm long). As the gaseous NH_3 passes through the denuder, it diffuses to the phosphorus acid-coated capillary walls, where it is removed. Using a test gas containing 7 ppm NH_3, we determined the NH_3 removal efficiency to be 99.9% at 21 l/min. In contrast, the particles do not diffuse rapidly and pass through the tubes where they are collected on filters for subsequent chemical analysis. In this way, the reaction between the NH_3 and the particles is stopped, and the chemical state of the particles exiting the absorbing device closely resembles the chemical state at the larynx.

Any difference between the chemical state of the particles on the filters and the state at the larynx is due to either size-dependent deposition of particles in the denuder or further chemical reaction with NH_3 either in the denuder or on the filters. Preferential gravitational deposition of larger, less neutralized particles in the denuder contributes only a small difference (less than a 2% overestimate of the extent of neutralization at the larynx for the relatively monodisperse size distribution ($\sigma_g < 1.2$) used in our experiments). Larger differences between the two chemical states can arise from additional reactions occurring either as the particles pass through the denuder or after they land on the filter. Based on tests of the denuder's performance, we estimate that the neutralization occurring in the denuder is equivalent to an additional 0.1 seconds of chemical reaction at the larynx. Contrasting the neutralization occurring in the denuder, there is the potential for re-acidification of particles on the downstream filters as NH_3 degasses from the particles into the essentially NH_3-free air passing through the filters. The magnitude of acidification is a function of the extent of neutralization of the particles on the filter, being greater for more neutralized particles. For our measurements there could be as much as a 10% underestimate of the extent of neutralization for 100% neutralized particles, but only about a 1% underestimate for 75% neutralized particles.

Particles exiting the denuder were collected on two nuclepore filters sampling in parallel for 40 minutes. On average, about 2 μg were collected on each filter. After sampling, one of the filters was exposed (for 15 seconds) to filtered air containing a concentration of approximately 100 ppm NH_3. Nuclepore filters do not absorb gaseous NH_3 and contain < 2 ng NH_3 per filter even after this NH_3 exposure. Therefore the NH_3 remaining after exposure is that which has reacted with acidic particles present on the filter. The amount of NH_4^+ on the unexposed filter divided by the amount on the exposed filter is the percentage neutralization of the sampled particles. The NH_4^+ mass was determined by placing the filter into 10 ml of 0.1 M NaOH containing $\sim 4 \times 10^{-6}$ M NH_4^+ and measuring the change in NH_4^+ concentration with a gas-sensing electrode. With this "known addition" technique, we can detect as little as 2 ng of NH_4^+ on the filter.

Based on eleven measurements of acidic aerosols in which both filters were exposed to excess NH_3, we have determined that the precision of this method in determining the overall extent of neutralization is ±5 percent deviation from the mean (mean value = 97% neutralization). The accuracy of this method has not been established because of the difficulty of preparing aerosols with exactly known $NH_4^+/SO_4^=$ ratios. The uncertainty arises from

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uncontrolled neutralization occurring during generation of the aerosol from the bulk solution. For instance, at the point of exposure of the dog, we measure an average of 8% neutralization for 0.5 um H_2SO_4 particles and 3% for 1.0 um particles produced by the aerosol generation system.

2.2 Results

Table 2.1 summarizes our measurements of NH_3 concentration in air sampled at the nose, mouth and trachea during quiet spontaneous breathing under anesthesia. Since the air is sampled continuously during both inhalation and exhalation, the NH_3 values do not represent those in expired air. Rather, they represent a time-weighted average of the value in expired air and the value in inspired air, i.e., the air that is immediately surrounding the point of sampling. We made no attempt to prevent rebreathing of expired air.

The mean NH_3 concentration in air sampled at the mouth is greater than that sampled at either the nose or trachea. In addition, there is no significant difference between the values obtained at the nose or trachea. We have compared our measured tracheal NH_3 concentrations with those expected if the air were in equilibrium with blood. Table 2.2 summarizes the measured values of ammonium ion concentration and pH in the dog's arterial blood as well as our estimate of the gaseous NH_3 concentration in equilibrium with the blood. The results summarized in Tables 2.1 and 2.2 are consistent with our earlier assertion (5, 6) that: 1) NH_3 concentrations in expired air are most strongly influenced by the last airway segment traversed; 2) the blood is the major source of NH_3 in the nose and trachea; 3) the mouth contains an additional source of NH_3. The fact that the NH_3 concentrations measured at the nose and trachea are lower than the blood equilibrium value is not surprising, since we did not selectively collect expired air. Our NH_3 levels are lower than those found by Jacquez and co-workers (10) who measured tracheal NH_3 concentrations in expired air in eight dogs. They found a mean value of 90 ppb (range 50 to 145 ppb) that was in equilibrium with the free NH_3 in arterial blood.

The measurements made at the nose, mouth and trachea during spontaneous breathing can be compared to the measurements made at the larynx during artificial, unidirectional flow through the isolated upper airways. Table 2.3 summarizes measurements made at the larynx on air leaving the larynx at 0.1 l/sec. The data are divided into those measurements taken when air entered via the mouth and those when air entered via the nose. The mean laryngeal NH_3 values are also independent of the route of entry even though (from Table 2.1) the nose and mouth passages contain different NH_3 levels. This result is consistent with our assertion that the NH_3 concentration is most strongly influenced by the most recent airway segments traversed by the air prior to sampling. In this case, the NH_3 levels in air exiting 3 tracheal rings below the larynx are most strongly influenced by common final pathways of the larynx and oro-pharynx, not by the more distant nose and mouth passages. However, this does not imply that our measured values are the same as those in the larynx and oro-pharynx. NH_3 can be absorbed by the walls of the trachea, and possibly the larynx, before the air reaches the sampling point.

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Table 2.1. NH_3 (ppb) in expired air of anesthetized dogs during quiet breathing†

--	Mean (±SE)	Range
Nose (n=7)	45 (±17)	19-75
Mouth	82 (±13)	40-133
Trachea* (n=8)	40 (±5)	15-68

† mean breathing rate = 9(±1) min^{-1}

* via tracheostomy

Table 2.2. Dog Blood Analyses

--	Mean (±SE)	Range
NH_4^+ (n = 8)	75 (±SE) µmoles/l	57-100
pH (n = 8)	7.32 (±0.02)	7.24-7.40
gaseous NH_3*	55 (±4) ppb	42-69

* calculated with human plasma equilibria at 38°C (13)

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Whereas the upper airway surfaces can either be a source or sink of NH_3 depending on the particular surface chemistry, these same surfaces are always a source of water vapor and heat during inhalation. Therefore the temperature and RH of the air exiting the larynx are integral properties, reflecting contributions from all the surfaces the air has contacted. The fact that the temperature and RH values in Table 2.3 are greater for passage through the nose than the mouth simply reflects the superior heat and mass transfer properties of the nose.

Like the concentration of water vapor, the extent of neutralization of acid particles in the airways is also an integral property reflecting the "dose" (NH_3 concentration multiplied by time) from all the airway segments traversed by the particle. Before discussing the results of our aerosol measurements, let us briefly examine those factors that influence the extent of neutralization of an acid particle. These include the relative amounts of NH_3 and H_2SO_4, the rate of reaction, and the time allowed for reaction. In our experiments, the most important of these factors is the rate of reaction. The factors that influence the reaction rate include the concentration of gaseous NH_3, the H_2SO_4 particle's proportion of surface area to acid mass, and the RH. The higher the NH_3 concentration, the more rapid the reaction rate. At a constant NH_3 level, smaller particles are more rapidly neutralized than larger ones. This occurs because smaller particles have more surface area exposed per mass of acid than larger ones. An increase in RH increases the particle size owing to accretion of water by hygroscopic growth. The particle's surface area increases but its acid mass does not. Therefore, increasing the RH at a given NH_3 concentration increases the rate of neutralization.

We found a linear relationship between the percentage neutralization and the NH_3 concentration measured at the larynx. Figure 2.2 shows this relationship for 0.5 um particles entering via the mouth. The neutralization values represent the difference between the percentage measured at the larynx and a value of 8% at the airway inlet. The neutralization values also include an additional 0.1 second of reaction time at the point of sampling due to the sampling procedures discussed earlier. The linear relationship implies that the laryngeal NH_3 concentration is proportional to the dose of NH_3 received by the particles. We can speculate that a majority of the neutralization is occurring in the larynx and oro-pharynx, passages where NH_3 levels are most likely proportional to laryngeal NH_3 and where the RH is high.

We observed large differences in both the NH_3 concentrations and percentage neutralization between different dogs but small differences within each dog on repeated measurements. Our measurements of human mouth NH_3 levels show similar inter and intra-subject variability (7). Table 2.4 shows the range of neutralization values observed for both particle sizes (0.5 um and 1.0 um) and both routes of entry (mouth vs. nose). In all cases, these results reflect differences between dogs. As expected, the percentage neutralization is less for the larger particles independent of the route of entry.

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Table 2.3. Measurements of Air Exiting the Larynx at 0.1 l/s.*

	ENTRY VIA MOUTH	ENTRY VIA NOSE
NH_3(ppb)	122 (± 14) n = 22	117 (± 15) n = 11
R.H. (%)	83 (± 0.9) n = 52	90 (± 0.6) n = 21
T (^oC)	33.3 (± 0.4) n = 52	36.3 (± 0.2) n = 21
ΔP(cm H_2O)	0.44 (± 0.07) n = 35	1.4 (± 0.15) n = 17
τ (sec)	--	0.73 (± 0.03) n = 12

* Mean ± standard error; n measurements on nine dogs

Table 2.4. Range of observed neutralization values.

	d_p = 0.5 µm	d_p = 1.0 µm
ENTRY VIA MOUTH	8 to 70% (n=16)	7 to 22% (n=6)
ENTRY VIA NOSE	15 to 63% (n=8)	16 to 18% (n=3)

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Table 2.5 shows the percentage neutralization per ppb of laryngeal NH_3 (the slope in Figure 2.2) for both particle sizes and routes of entry. These values reflect both the average rate of reaction and the residence time in the airways. The values are independent of aerosol mass concentration, implying negligible depletion of the gaseous NH_3 surrounding each particle. For entry via the mouth, the value is greater for the 0.5 um particles than for the 1.0 um particles. For the 0.5 um particles, the percentage neutralization per ppb of laryngeal NH_3 is larger when the particles enter via the mouth than when they enter via the nose (difference significant at $p < 0.01$). This result implies that the particles passing through the nose received a lower dose of NH_3 than those passing through the mouth, a result consistent with our observation (Table 2.1) that the NH_3 levels in the nose of the dog are lower than those in the mouth.

In one dog, we have made several preliminary measurements of temperature, RH, NH_3 and aerosol neturaliztion at one of two different flow rates through the mouth, either 0.1 or 0.2 l/sec. We changed the particle size from 0.5 um to 0.7 um because of the difficulty of producing 100 ug/m^3 of 0.5 um particles at 0.2 l/sec. The measurements are listed in Table 2.6. We did not measure the residence time. As expected, the NH_3 level, RH and percentage neutralization decrease at the higher flow rate. Allowing for the small number of measurements, the percent neutralization per ppb NH_3 also appears to decrease, reflecting a decrease in residence time and RH.

For a given particle size, NH_3 concentration, relative humidity, temperature and residence time we can calculate the theoretical percentage neutralization of a H_2SO_4 particle. Our calculation assumes that the reaction rate is controlled by gaseous diffusion to the particles surface and that the parameters just mentioned are constant. This latter assumption reflects our lack of knowledge of the temperature, RH and NH_3 concentration profiles in the airways. Our simplified model produces the following expression for the percentage neutralization, χ:

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$$\chi = 100(6/d^{-2}\ D\ C_N\ \alpha\tau\ [SO_4^{=}]^{-1})$$

where:

d = particle diameter at the given R.H. (cm)

D = diffusivity of NH_3 (cm^2/sec)

C_N = concentration of NH_3 (moles/cm^3)

$\alpha = (1 + (1/\ _r\ -1)\ 4Kn\ f\ (Kn)/3)^{-1}$

α_r = condensation coefficient (= 0.25 for H_2SO_4 - NH_3 (reference 12))

Kn = 2 λ/d = particle Knudsen No.

λ = mean free path of air molecules (1.1×10^{-5}cm @ 33°C)

$f(Kn) = (1 + Kn)(1 + 1.71\ Kn + 1.333\ Kn^2)^{-1}$

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τ = residence time (sec)

$[SO_4^{=}]$ = concentration of $SO_4^{=}$ dissolved in drop at given RH (moles/cm^3).

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Assuming a flow through the nose at an RH of 90%, a temperature of 36°C, a particle size of either 0.85 um or 1.7 um @ 90% RH (equivalent to 0.5 um and 1.0 um respectively @ 10% RH), and a residence time of 0.8 seconds (0.7 seconds in the airways and 0.1 seconds in the denuder), we arrive at a value of 0.14% (±0.05%) neutralization per ppb NH_3 for the 0.5 um particles. The calculated uncertainty reflects our lack of knowledge of the exact particle size and residence time. This can be compared with the measured value of 0.19% per ppb (Table 2.5). Our model also predicts that the percent neutralization per ppb NH_3 for 1.0 um particles is 32% of that for 0.5 um particles. This can be compared with an average measured value of 21% for flow through the mouth. The agreement between our predicted and measured values is reasonable considering the simplicity of our model.

Using this simplified model we have extrapolated our results to humans. For a range of mouth NH_3 concentrations about the mean of 700 ppb (7), a residence time of 0.5 seconds, an RH of 90% and a flow rate of 0.1 l/sec through the mouth, we arrive at the laryngeal neutralization values shown in Figure 2.3 as a function of initial particle size (at 50% RH) and NH_3 concentration. The values apply to mouth breathing; breathing by nose would lower our estimate of neutralization by approximately a factor of two.

These results suggest that H_2SO_4 particles with diameters less than 0.5 um (at 50% RH) that are inhaled via the mouth will be fully neutralized by NH_3 prior to their reaching the trachea. Particles with larger diameters may or may not be fully neutralized, depending on the relative humidity and NH_3 concentrations they encounter in the mouth and oro-pharynx.

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Table 2.5. Percent neutralization per ppb laryngeal NH_3

	d_p = 0.5 μm	d_p = 1.0 μm
ENTRY VIA MOUTH	0.28 (0.08)	0.06 (0.06)
ENTRY VIA NOSE	0.19 (0.21)	---

( ) = 95% confidence limits

Table 2.6. Laryngeal Measurements on One Dog as a Function of Flow Rate Through the Mouth.

	FLOW = 0.1 l/sec	0.2 l/sec
Temperature (^oC)	35;35;36	32;31;32
Relative Humidity (%)	76;76;82	60;47;46
NH_3(ppb)	121;124	74;50
% Neutralization+	19;24	9;3
% per ppb NH_3*	0.16;0.19	0.12;0.06

\+ particle diameter = 0.7 μm @ 20% RH

* calculated from above data

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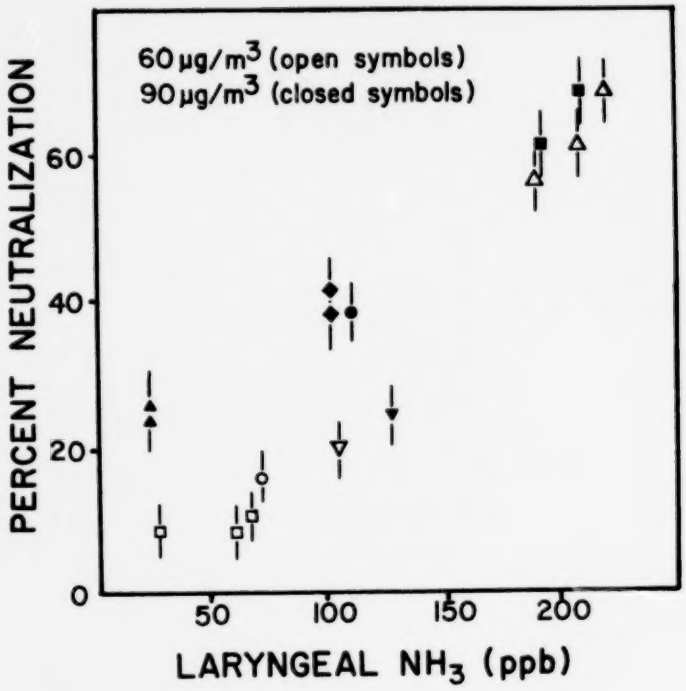
Figure 2.2. Neutralization of 0.5 μm H_2SO_4 particles by NH_3 in nine dogs (mouth → larynx, 0.1 l/sec).



32°C < T < 37°C, 70% < RH < 90%
Symbols refer to different dogs

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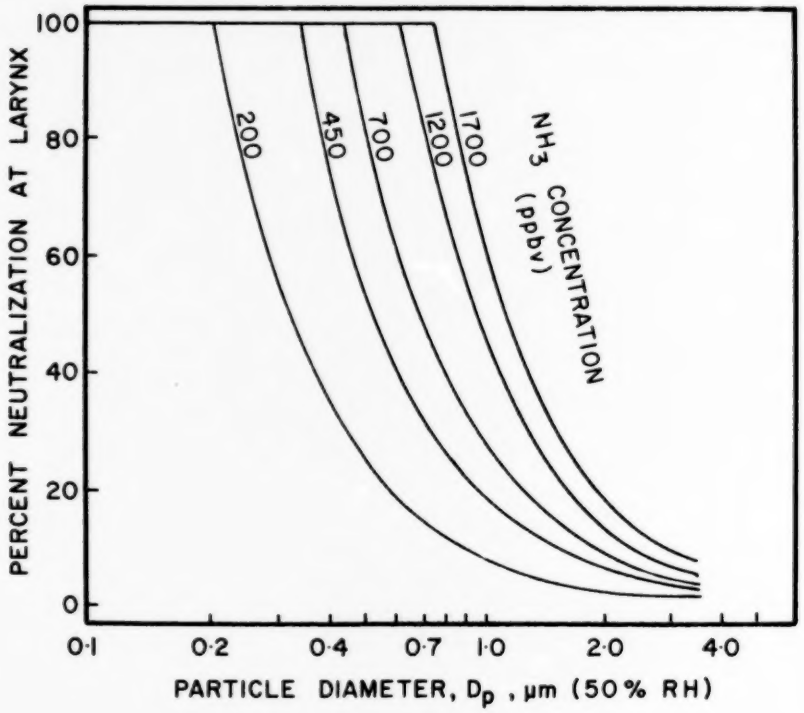
Figure 2.3. Calculated neutralization of inhaled H_2SO_4 particles in humans during mouth breathing.



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2.3 Conclusions

We have shown that H_2SO_4 particles can undergo significant neutralization during inhalation via the nose or mouth by the time the particles pass the larynx. For a given flow rate, the percent neutralization depends both on NH_3 concentration and particle size, with 0.5 um particles being neutralized more rapidly than 1.0 um particles. Our measurements of the rate of neutralization are in reasonable agreement with simple diffusion theory.

On the basis of chemical transformation occurring in the airways, our results suggest that smaller acid particles may be less irritating than larger ones and that inhalation via the nose is not necessarily more protective than via the mouth. These conclusions are tempered by the fact that the irritant potential of an acid particle is determined not only by its chemistry but also by its site of deposition. Nevertheless, our results can partially explain the results of Wolff and coworkers (14), who found that 0.9 um H_2SO_4 particles are more irritating to dogs than 0.3 um particles, but cannot help to explain the results of Amdur and co-workers (15), who found that 0.3 um H_2SO_4 particles were more irritating to guinea pigs than the same concentration of 1.0 um particles. In conclusion, neutralization of H_2SO_4 aerosol by airway NH_3 appears to be an important phenomena that can be studied by relatively simple techniques.

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2.4 REFERENCES

1. Charlson, R.J., Vanderpol, A.H., Covert, D.S., Waggoner, A.P., Ahlquist, N.C. (1974): Sulfuric acid - ammonium sulfate aerosol: optical detection in the St. Louis region. Science 184: 156-158.

2. Brosset, C., Andreasson, K., Ferm, M. (1974): The nature and possible origin of acid particles observed at the Swedish west coast. Atmos. Environ. 9: 621-642.

3. Cunningham, P.T., Johnson, S.A. (1976): Spectroscopic observation of acid sulfate in atmospheric particulate samples. Science 191: 77-79.

4. Lippman, M., Albert, R.E., Yeats, D.B., Wales, K., Leikauf, G. (1980): Effect of sulfuric acid mist on mucocilliary bronchial clearance in healthy non-smoking humans. J. Aerosol Sci.: in press.

5. Larson, T.V., Covert, D.S., Frank, R., Charlson, R.J. (1977): Ammonia in the human airways: neutralization of inspired acid sulfate aerosols. Science 197: 161-163.

6. Larson, T.V., Covert, D.S., Frank, R. (1978): Respiratory NH_3: a possible defense against inhaled acid sulfate compounds. In Folinsbee, L.S. (ed): "Environmental Stress: Individual Human Adaptations". New York: Academic Press: 91-99.

7. Larson, T.V., Covert, D.S., Frank, R. (1979): A method for continuous measurement of ammonia in respiratory airways. J. Appl. Physiol. 46 (3): 603-607.

8. Cobourn, W.G., Djukic-Husar, J., Husar, R.B. (1980): Monitoring of sulfuric acid episodes in St. Louis, Missouri. JGR Oceans and Atmos.: in press.

9. Pierson, W.R., Brachaczek, W.W., Truex, T.J., Butler, J.W., Korniski, T.J. (1980): Ambient sulfate measurements on allegheny mountain and the question of atmospheric sulfate in the Northeastern United States. Annal NY Acad. Sci. 338: 145-173.

10. Jacquez, J.A., Poppell, J.W., Jeltsch, R. (1958): Partial pressure of ammonia in alveolar air. Science 129: 269-270.

11. Fuchs, N.A., Stitugin, A.G. (1971): High-dispersed aerosols. In Hidy, G.M. and Brock, J.R. (ed.): "Topics in Current Aerosol Research" 2: Pergamon Press, Oxford: 36.

12. Huntzicker, J.J., Cary, R.A., Ling, C. (1980): Neutralization of sulfuric acid aerosol by ammonia. Env. Sci. Tech.: in press.

13. Jacquez, J.A., Poppell, J.W., Jeltsch, R., (1959): Solubility of ammonia in human plasma. J. Appl. Physiol. 14: 255-258.

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14. Wolff, R. (1980): Effects of H_2SO_4 mist on tracheomucus clearance in awake beagle dogs. Inhalation Toxicology Research Institute, Albuquerque, New Mexico, Annual Report.

15. Amdur, M.O., Dubriel, M., Creasia, D.A. (1978) Respiratory response of guinea pigs to low levels of sulfuric acid, Env. Res. 15: 418-423.

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SECTION 3

THE RESPIRATORY RESPONSE OF GUINEA PIGS TO AEROSOLS: I HISTAMINE AEROSOL CONTROL STUDY

The investigation of the potentially mild pulmonary functional responses in guinea pigs to components of atmospheric aerosols requires an accurate description of both the aerosol under investigation (chemical species, mass concentration and size distribution of the droplet or particle, temperature, relative humidity) and of the animal preparation. The use of guinea pigs is confounded by intra and inter-animal variability and small but important differences in protocols between laboratories which make comparisons difficult.

This paper describes the time course of pulmonary functional response (dynamic compliance and flow resistance) in guinea pigs exposed to moderate concentrations, 565 and 885 ug/m^3 of histamine aerosol, a known bronchoconstrictor. The aerosol parameters and the sensitivity of the guinea pig preparation are sufficiently well described to permit interlaboratory comparison. Our results will provide a basis for a forthcoming paper on the analysis of pulmonary functional response data of guinea pigs that were exposed to aerosols of SO_2 and particulate sulfate compounds.

3.1 Materials and Methods

Female guinea pigs (301-305g Charles River Hartley Strain, COBS) were monitored for dynamic lung compliance (C_{dyn}), pulmonary flow resistence (R_L), breathing frequency (f) and minute volume (V_{min}). The method described by Amdur and Mead (1) was used with the following modification: Ketamine hydrochloride (Parke-Davis, 21-25 mg/kg) and sodium pentobarbital (Abbott, 21-30 mg/kg) were administered (I.M.) to anesthetize the animal for insertion of the pleural pressure catheter. Animals were then allowed to recover partially, that is, until f reached 50-60 breaths per minute before the experiment was initiated; this corresponded to 65 min after anesthetics were administered.

Histamine dihydrochloride aerosol was generated by passing air through a Corning 12c fritted glass bubbler in a 5% by weight solution of histamine dihydrochloride (Sigma Chem). The aerosol was mixed turbulently with filtered, humidified air in an all glass chamber with a residence time of ~ 70 sec. The aerosol was monitored continuously for temperature and dew point temperature (EG & G dew point hygrometer model 880) and light scattering coefficient (MRI 1550 integrating nephelometer) immediately upstream of the head chamber of the animal. Primary values of mass

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concentration were obtained with filter samples (Nuclepore polycarbonate membranes, 0.4 um pore size) and size distribution of the aerosol was determined with an optical particle counter (0.25 um to 10.0 um diameter). The histamine droplet aerosol had a mass median diameter of 0.65 um, (σ_g = 1.4). Temperature was maintained at 21-22°C and relative humidity at 75-80%. Each guinea pig was studied at one of two aerosol concentrations: 565 ug/m^3 (range 539-586, n=6) or 885 ug/m^3 (range 771-956, n=10).

For completeness, we include here a description of the guinea pig body plethysmograph used in the study and steps taken to identify and eliminate artifacts in the data due to flow dynamics in the plethysmograph:

1. We have found that a plethysmograph with a configuration which permits the animal's fur to touch the sides of the box reassures the animal, reducing struggling and random movements. The plethysmograph is cylindrical with an 8 cm I.D. and length of 30 cm. A 3 liter reservoir filled with copper wool is attached to reduce the work of breathing upon the air in in the plethysmograph and to insure isothermal gas compression. We have calculated the work done on the box by the guinea pig during breathing to be 60 g-cm/min. This is ~5% of the total work of breathing we calculate from our typical values of C_{dyn}, R_L, f, tidal volume (V_t), as well as with other published values (2).

2. To insure that artifacts were not being introduced by leaks within the plethysmograph, the time constant for decay of a step change in pressure was measured and found to be ~1.5 sec. Since the time for a single breath of the guinea pig is 0.3 - 0.5 sec, the decay constant is a factor of 3 to 5 greater than the event time. An additional leak is possible around the ensolite neck seal of the guinea pig; this is checked for by examining the frequency dependence of a constant volume signal (from an animal respirator) at the completion of each experiment, after the animal is dead but still in the neck seal.

3. No discernible time lag was observed in transmission of the volume signal from the plethysmograph to the transducer using 0.6 cm ID x 46 cm long tygon tubing as a connector. We estimate the phase lag angle to be < 1.5°.

4. We have also tested the time for transmission of the pleural pressure signal. For the liquid-filled polyethylene tubing that we use (Intramedic #7416, PE 60, 0.030" ID, 0.048" OD), we have found that in

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lengths less than or equal of 46 cm, the transmission lag appeared to be 5 to 10 msec. We record pleural pressure (P_{pl}), Tidal Volume, (V_t), and flow, (V) at a chart speed of 25 mm/sec and can expect resolution to 1/ mm (i.e., 20 msec.); therefore, the transmission lag is 2 to 4 times shorter than uncertainties in the calculation.

Since our preparation differs from the original Amdur and Mead preparation in the choice of anesthetic (originally ether) used to allow implantation of the catheter, we felt it useful to study the effect of histamine on the guinea pigs at two levels of anesthesia.

The experiment consisted of two 10-minute histamine aerosol exposure periods separated by one hour; P_{pl}, V_t, V, and f were monitored each minute for 10 minutes prior to and during the 10-minute exposure periods. C_{dyn} and R_L were calculated from two successive breaths randomly chosen within each interval, except for the requirement that the breaths used for calculation be separated from a deep inspiration by five breaths. The one hour between exposures was designed to allow recovery from the first exposure and to allow the guinea pig to reach a lighter level of anesthesia.

Each guinea pig served as its own control, permitting us to analyze for changes in C_{dyn}, R_L in both the individual animal and the group of animals. We used the Walsh test (10) to assess changes within the guinea pig by comparing the ten one-minute interval control values with the ten one-minute exposure values. This non-parametric test was used since the data derived from closely-spaced sampling intervals cannot be assumed to be independent. A significant response was defined as a change in C_{dyn} or R_L at $P \leq 0.05$. Results are summarized in Table 3.1.

The paired t-test (13) was used to assess the changes in each group of animals; average values of C_{dyn} and R_L for the control and exposure periods were paired for this test. The results are summarized in Table 3.2.

3.2 Results and Discussion

The only persistent change observed in any guinea pig exposure mode was the decrease in C_{dyn} (statistically significant in nine of ten animals), we examined the time course of response to this aerosol. The average intra-animal coefficient of variation of C_{dyn} during control periods was 9% and the average inter-animal coefficient of variation of C_{dyn} was 30%; this is very close to the findings of Dennis, et al. (5). The large inter-animal variation adds dispersion to the data not related to observations on the time-course effect of histamine aerosol; this variation was eliminated by "normalizing" the data for each guinea pig. The values of C_{dyn} for each guinea pig during the control and exposure periods were divided by the average control value for that guinea pig. These normalized data points were pooled and plotted versus time in Figure 3.1. The

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vertical bars represent one standard deviation for each pooled data point for the ten guinea pigs. A progressive decrease in C_{dyn}, which began within one minute, accompanied exposure to histamine. After 5-6 min, dispersion in the data increased. This can be explained, at least in part, by the large number of deep breaths observed (5).

There has been discussion (3,6,11) of the hypersensitive fraction of the guinea pig population. To explore this idea, data from guinea pigs which had been exposed to 565 ug/m^3 with significant changes in C_{dyn} ($p < 0.05$, Walsh test) during the first exposure were normalized and pooled in the same manner described above. The time course of response at the two concentrations was superimposed in Figure 3.2. Although the lower concentration contains data for only three animals, the results are consistent with the idea that a few individuals may respond in the same manner as the general population, but at a lower concentration.

Guinea pigs which responded to the second exposure of 565 ug/m^3 histamine aerosol exhibited the same pattern of response; two of the three responding guinea pigs were in the responding group for the first exposure. When the guinea pigs which responded to the first exposure at this concentration were normalized and pooled for the second exposure, the pattern of response was similar but more erratic.

Only 5 of the 10 guinea pigs responded to the second exposure of 885 ug/m^3 histamine aerosol; the pattern of response did not exhibit the progressive decrease in C_{dyn} throughout exposure. Douglas et al. (8) and Silbaugh et al. (12) indicate that with short (<2 min) exposures, recovery occurs within 15-30 min. Our results indicate that with 10 min exposures, either recovery may take longer or there may be some adaptation which modifies the second response.

The guinea pigs reached a lighter level of anesthesia between exposures if judged by the average frequency of breathing (f = 62) for first control period, f = 76 for second control period). Although the average values of C_{dyn} were within 10% of the original control, the second exposure results for the higher concentration appear masked by either adaptation or incomplete recovery after the first ten-minute exposure. The effects of repeat exposure to the higher concentrations of histamine were not clear, but there was no increase in the number of responding guinea pigs or in the magnitude of response for animals receiving the lower concentration a second time; we therefore tentatively conclude that the initial anesthetic does not dull the pulmonary functional response to histamine aerosol.

Table 3.3 lists the average values of C_{dyn}, R_L, V_{min}, and f of our guinea pigs for control and exposure periods. Also included are baseline values of Amdur and Mean (1), Stein (14), Dennis et al. (5), and Silbaugh et al. (11), preparations where the guinea pig is considered to be unanesthetized.

The unanesthetized guinea pig is reported to have a breathing frequency of 60-90 breaths per minute (9). The average breathing frequency we observed was within this range, though somewhat lower than those listed in

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the table. The minute volume was markedly lower. Average C_{dyn} is consistent with those found by Amdur and Mead, Stein, and Dennis, et al., but lower than than found by Silbaugh et al. Average R_L is consistent with Silbaugh et al. and Dennis et al., but lower than found by Amdur and Mead and Stein. The implication of these data is that the guinea pigs in our preparation are anesthetized sufficiently to depress minute volume but not enough to affect normal values of C_{dyn} and R_L.

As can be seen in Figure 3.1, at an exposure concentration of 885 ug/m^3, C_{dyn} decreased by 10-15% in the first two to three minutes and decreased by 40% by the end of the ten-minute exposure. The average decrease over the entire exposure period was 27%. Silbaugh et al. (11) exposed guinea pigs to histamine in a stepwise manner. With an initial concentration of 20 ug/m^3, guinea pigs were repeatedly exposed for two minutes with a doubling in aerosol concentration until C_{dyn} decreased by 50%. Eighteen of twenty-two animals were found to respond at 440 g/m^3, which corresponded to a total exposure period of fifteen minutes. Amdur (4) exposed guinea pigs to 3000, 6000, 9000 ug/m^3 for twenty-five minutes. At the lowest exposure concentration, the data indicate a decrease of 27% at the end of 10 minutes of exposure, with an average decrease of 32% over the entire 25-minute exposure. These findings indicate that the response to histamine aerosol (as measured by change in C_{dyn}) found in our lightly anesthetized guinea pigs is within the range of response of animals which are considered unanesthetized; we would judge our preparation somewhat less responsive than one preparation discussed (11) and at least as sensitive as another (4).

Changes in C_{dyn} without changes in R_L at low concentrations are consistent with previous studies. Silbaugh et al. (11) found changes in C_{dyn} alone at concentrations of 400-800 ug/m^3. Amdur (4) found changes in both C_{dyn} and R_L at concentrations of 3000 to 9000 ug/m^3. Douglas et al. (7) found changes in C_{dyn} alone at lower concentrations and changes in both C_{dyn} and R_L at higher concentrations. This study therefore does not permit us to make conclusions about the sensitivity to change in R_L of our animal preparation.

We have monitored C_{dyn} and R_L for exposures of guinea pigs to mixtures of SO_2 and sulfate compounds [H_2SO_4, $(NH_4)_2SO_4$, Na_2SO_4]. These results will be presented in a future paper. Any findings of significant changes in C_{dyn} and R_L will be accompanied by a discussion of the relative sensitivity of these parameters in our preparation compared to other exposure data available in the literature via the histamine control data presented here.

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Table 3.1. Walsh Test Results $p \leq 0.05$

Histamine Mass Concentration	C_{dyn}	R_L
565 µg/m^3 (n = 6)	1st exposure: 3 significant decreases 2nd exposure: 3 significant decreases, 1 significant increase	1st exposure: 2 significant decreases 2nd exposure: 2 significant decreases, 1 significant increase
885 µg/m^3 (n = 10)	1st exposure: 9 significant decreases 2nd exposure: 5 significant decreases, 1 significant increase	1st exposure: 3 significant increases 2nd exposure: 2 significant increases, 1 significant decrease

Table 3.2. Paired t Test Results

Histamine Mass Concentration	C_{dyn}	R_L
565 µg/m^3 (n = 6)	1st exposure: t = 1.95 2nd exposure t = 1.39	1st exposure: t = 1.22 2nd exposure: t = 1.02
885 µg/m^3 (n = 10)	1st exposure: t = 5.90 ($p \leq 0.001$) 2nd exposure: t = 1.34	1st exposure: t = 0.94 2nd exposure: t = -1.12

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Table 3.3 Average values of pulmonary functional measurements; values obtained in this histamine exposure study and control values of other investigators.

	885 $\mu g/m^3$ exposure (n = 10)	565 $\mu g/m^3$ exposure (n = 6)	Combined (n = 16)
Body weight			301-350g
C_{dyn}			
1st control	.21 ± .06	.19 ± .06	.20 ± .06
1st exposure	.15 ± .05	.16 ± .04	
2nd control	.19 ± .05	.19 ± .07	
2nd exposure	.18 ± .05	.16 ± .04	
R_L			
1st control	.27 ± .13	.36 ± .40	.30 ± .25
1st exposure	.25 ± .12	.30 ± .29	
2nd control	.32 ± .14	.38 ± .31	
2nd exposure	.33 ± .14	.27 ± .11	
f			
1st control	66 ± 18	55 ± 19	62 ± 18
1st exposure	83 ± 20	62 ± 17	
2nd control	79 ± 16	69 ± 18	76 ± 17
2nd exposure	86 ± 18	78 ± 15	
V_{min}			
1st control	104 ± 23	79 ± 21	95 ± 25
1st exposure	112 ± 23	88 ± 26	
2nd control	122 ± 21	105 ± 13	
2nd exposure	121 ± 22	107 ± 17	

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Table 3.3 Continued

	Silbaugh (n = 10)	Dennis Douglas (n = 9)	Amdur (n = 200)	Stein (n = 10)
	350-460g	250-350g	130-290g	210-270g
C_{dyn}	.60 ±.17	.16 ±.04	.20 ±.05	.23 ±.01
R_L	.29 ±.08	.34 ±.09	.73 ±.21	.63 ±.02
f	.104 ±17		84 ±14	90 ±5
V_{min}	281 ±43		139 ±30	164 ±6

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Figure 3.1. Time course of C_{dyn} during control and exposure periods. Normalized data; exposure concentration 885 $\mu g/m^3$.

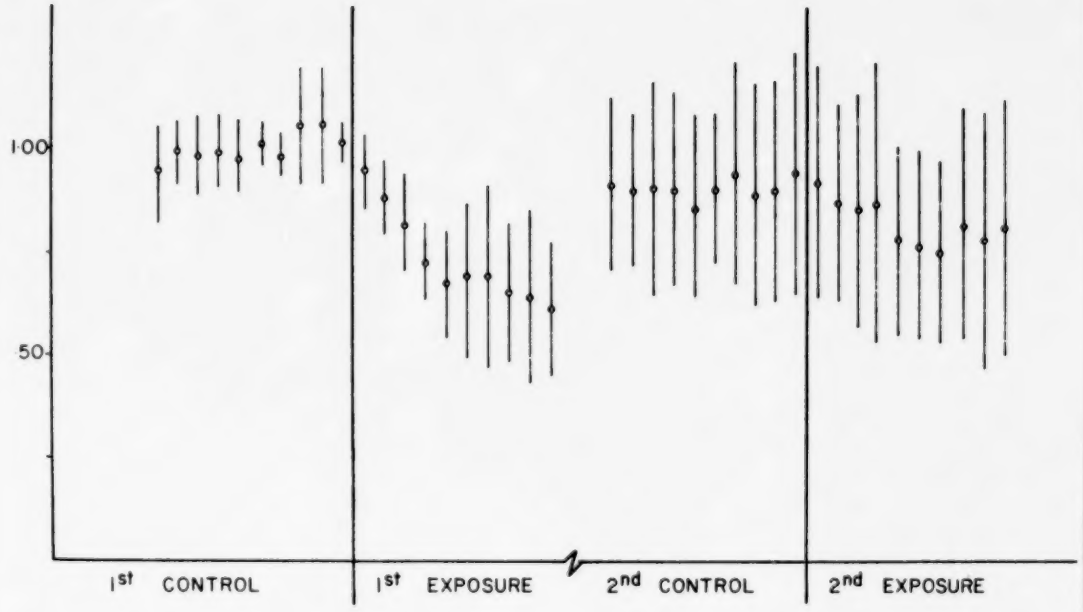

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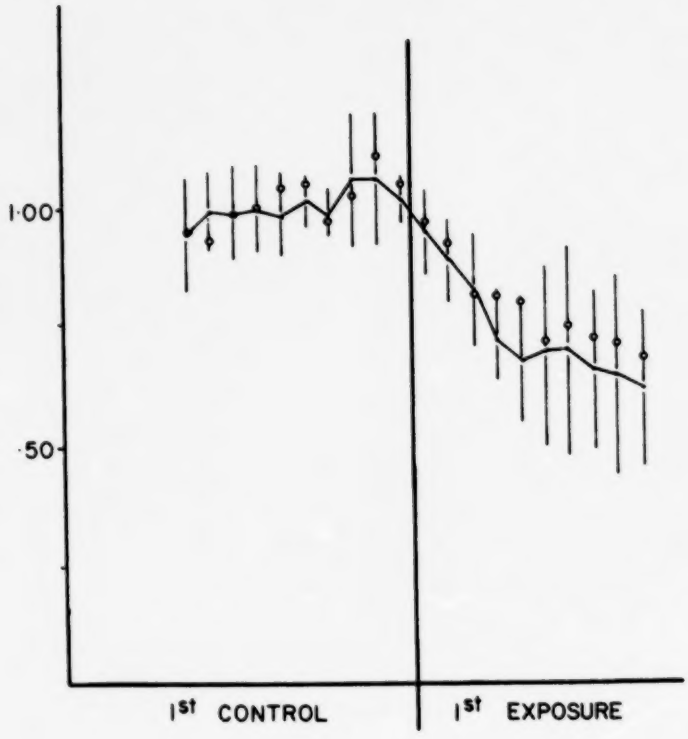
Figure 3.2. Time course of C_{dyn} for responding guinea pigs (Walsh test) at 565 µg/m^3 superimposed on 885 µg/m^3 exposure data.



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3.3 REFERENCES

1. Amdur, M.O., Mead, J. (1958): Mechanics of respiration in unanesthetized guinea pigs, Am. J. Physiol., 192(2), 364-368.

2. Amdur, M.O. (1958): The respiratory response of guinea pigs to sulfuric acid mist, Arch. Ind. Health, 18: 412.

3. Amdur, M.O. (1964): The effect of high flow resistance on the response of guinea pigs to irritants, Amer. Ind. Hyg. Assoc. J., 25, 564-568.

4. Amdur, M.O. (1966): The respiratory response of guinea pigs to histamine aerosol, Arch. Environ. Health, 13: 29-37.

5. Dennis, M.W., Douglas, J.S., Casby, J.U., Stolwijk, J.A.J., Bouhuys, A. (1969): On-line analog computer for dynamic lung compliance and pulmonary resistance, J. Appl. Physiol., 26, 248-252.

6. Dennis, M.W., Douglas, J.S. (1970): Control of bronchomotor tone in spontaneously breathing, unanesthetized guinea pigs, Airway Dynamics, Physiology and Pharmacology, Charles C. Thomas, Publisher, Springfield, Ill., 253-262.

7. Douglas, J.S., Ridgway, P., Dennis, M.W. (1969): Histamine release by air pollutants, Arch. Environ. Health, 18: 627-630.

8. Douglas, J.S., Dennis, M.W., Ridgway, P., Bouhuys, A. (1972): Airway dilation and constriction in spontaneously breathing guinea pigs, J. Pharmacol. Exp. Ther., 180: 98-109.

9. Mead, J. (1960): Control of respiratory frequency, J. Appl. Physiol., 15: 325-336.

10. Siegel, S. (1956): Nonparametric statistics for the behavioral sciences, McGraw-Hill, NY, pp. 83-87.

11. Silbaugh, S.A., Wolff, R.K., Carpenter, R.L., Mauderly, J.L. (1976-1977): Effects of sulfuric acid and histamine aerosols on pulmonary function of guinea pigs, Inhalation Toxicology Research Institute Annual Report, pp. 429-434.

12. Silbaugh, S.A., Personal Communication

13. Snedecor, G.W. (1956): Statistical Methods, Iowa State University Press, Ames, Iowa, p. 46.

14. Stein, M., Schiavi, R.C., Ottenberg, P., Hamilton, C. (1961): The mech. properties of the lungs in experimental asthma in the guinea pig, J. Allergy, 32:8-16.

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SECTION 4

THE INFLUENCE OF EXERCISE AND ENDOGENOUS AMMONIA ON PULMONARY IRRITATION BY SULFURIC ACID AEROSOL

The occurrence of oxidized sulfur species in the atmosphere is a well-known consequence of fossil fuel combustion. Depending on local conditions, these species may include the acid sulfates ammonium sulfate ($(NH_4)_2SO_4$), ammonium bisulfate (NH_4HSO_4) and sulfuric acid (H_2SO_4) (7, 19). Human exposure to these compounds is likely to increase as coal utilization rises, and their effect on respiratory function must be clarified if appropriate controls are to be devised. Based upon toxicity experiments in guinea pigs, Amdur and associates (2) have suggested that, in relative irritant potency, H_2SO_4 exceeds the other sulfates by a considerable margin. Only a few controlled exposures of human subjects to acid sulfates have been reported, however, and the results show that in general, acid sulfate concentrations ranging up to the known worst case levels produce no changes in lung mechanical function.

Recently, Larson and associates (12) identified a factor which may be of considerable importance in determining human response to inhaled acid particles. They showed that ammonia (NH_3) generated within the normal airways, and especially in the mouth, is capable of converting inhaled sulfuric acid to $(NH_4)_2SO_4$ or to NH_4HSO_4 and presumably reducing its irritant potency. The authors calculated that ammonia produced in the mouth was sufficient to convert up to 1500 ug/m^3 H_2SO_4 to $(NH_4)_2SO_4$, a nearly neutral salt, provided that the acid particles remained in the mouth for a sufficient time. That this occurs while the acid particles traverse the mouth before entering the lower airways could not be confirmed, but full conversion was found in exhaled particles. It was proposed that inhaled acid particles may not be fully neutralized before entering the lower airways under certain conditions, namely: during nose breathing because of lower NH_3 concentrations, and during rapid mouth breathing when particles encounter high NH_3 levels only briefly. Under these conditions, evidence of lower airways irritation might be expected, while during exposure with quiet mouth-breathing, no effect was anticipated.

To test a part of this hypothesis, human subjects were exposed to sulfuric acid particles during mouth breathing while at rest and then while exercising. The particle concentration, particle size and exercise level were chosen so that neutralization in the mouth should not be complete during exercise, while at rest the conversion was expected to be more nearly complete. Lung mechanical function was determined before and after exposure both at rest and with exercise.

4.1 Materials and Methods

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Subjects: Ten males ranging in age from 21 to 57 were recruited for the study. All denied a history of respiratory disease, including asthma, and were free of acute respiratory symptoms for at least one month prior to the study. Informed consent was obtained from each.

NH_3 measurements: While mouth breathing, exhaled air was sampled for NH_3 using the method described by Larson, et al.(13) in which a portion of exhaled air was bubbled through a H_2SO_4 solution and the NH absorbed was quantitated by a specific ammonium ion electrode. Exhaled NH_3 was determined before and after each experiment.

Aerosol generation: We selected a concentration of 500 g/m^3 sulfuric acid. This is within the range of previous human exposure studies (4,17,15,1)) and although it exceeds current "worst case" ambient levels in the U.S., this concentration is reported in certain industrial environments (3). This amount of H_2SO_4 could be fully converted to $(NH_4)_2SO_4$, provided sufficient time, by 167 ug/m^3 NH_3, which is less than half the median mouth value reported (13).

The aerosol was produced by a generator described previously (11). Filtered, compressed air was bubbled through 5% (wt) aqueous H_2SO_4 and the resulting particles were diluted with a second stream of filtered air. Water vapor was added and the mixture was allowed to equilibrate at room temperature (21C) in a teflon-lined chamber. The total flow rate was 240 liters (STP)/min.

The relative humidity (RH) was adjusted to 75 + 2%, at which condition the H_2SO_4 forms droplets which are 32% (wt) acid (16). Thus, at 500 ug/m^3 H_2SO_4, the total suspended particle concentration was 1500 ug/m^3. The aerosol light scattering coefficient was monitored during exposure using an integrating nephelometer calibrated for mass concentration as follows: aerosol droplets were collected on a Nuclepore filter and converted to $(NH_4)_2SO_4$ by exposure to the vapor over a saturated solution of NH_4OH. At room relative humidity, this salt rapidly dehydrates, so that the mass of sulfate is determined, and quantity of H_2SO_4 is calculated. The nephelometer calibration was also checked after each exposure by the same method. During the ten exposures, the H_2SO_4 concentration was 500 ± 25 ug/m^3.

The particle size distribution was determined using an optical particle counter and an automated pulse height analyzer. The particle mass median diameter at 75% RH was 0.65 um, with a geometric standard deviation of 2.2. This size distribution closely approximates the ambient air pollution accumulation mode in which H_2SO_4 is found (20). Within the airways the particles will grow as the temperature and RH rise. Assuming conditions of 33C and 90% RH in the oropharynx, the particles will have a median

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equilibrium diameter of 0.76 um. The time required to fully neutralize the particles was estimated using the diffusion model described by Larson, et al.(12). For mouth NH_3 concentrations of 200-700 ug/m^3, the times were 0.8-0.1 sec. During resting mouth breathing, we estimate that the mean residence time in the mouth should be no more than 0.8 sec, while on exercise at the levels used here the corresponding time is 0.2 sec or less. Thus neutralization should be nearly complete at rest, but a significant portion of the H_2SO_4 should enter the trachea unconverted during exercise.

Exposure protocol: Subjects breathed the aerosol via a rubber mouthpiece while wearing a nose clip. When at rest, the subjects sat erect, and during exercise they walked on a treadmill at a speed and grade sufficient to increase minute volume to four times the resting value. Resting exposures lasted thirty minutes and exercise exposures lasted ten minutes. Prior to exposure, the subjects rested for thirty minutes and then exercised for ten minutes before baseline measurements were made. Two sets of rest-exercise exposures followed, making a total exposure of eighty minutes. Each subject was studied on two days at least one week apart, receiving sham (filtered air, 75% RH) and acid exposures in random order. In this design, two sets of controls were provided: the daily baseline was used to determine the relative effect of exposure on that day, while the sham exposure revealed the effects of time and exercise, independent of exposure to H_2SO_4.

Functional measurements: After each rest or exercise session, total respiratory flow resistance (R_{rs}) was determined by imposing small pressure oscillations at 3 Hz at the mouth. The apparatus was similar to that described by Sackner, et al.(17). R_{rs} was calculated from data obtained only during early respiration, when the glottis imposed least interference (18). Following this, three partial forced expiratory maneuvers were initiated from about one liter above functional residual capacity. Each was followed by a maximum inspiration and one minute's rest. From these data, maximum flow at 50% and 75% expired vital capacity (V_{max50} and V_{max75}) were determined. After each exercise session, in addition to the above data, a multiple breath nitrogen wash-out was performed. From a semi-logarithmic plot of exhaled nitrogen concentration versus cumulative expired volume, the slope and regression coefficient were determined, as a measure of uniformity of regional ventilation.

At the start and finish of each day's session, after the exhaled NH_3 measurement, the density dependence of maximum expiratory flow was determined. After equilibrating on 80%He-20%O_2, subjects performed three maximum expiratory maneuvers. Flow rates were measured at 50% and 75% expired vital capacity, and these were compared to similar values from preceding maneuvers done on air. Density dependence was defined as the ratio of maximum flow on He-O_2 to that on air at each expired volume (DD_{50} and DD_{75}). In addition, the volume at isoflow (V_{isov}) was determined by superimposing the flow-volume curves representing the best efforts on He-O_2 and air. Change in density dependence has been shown to be a sensitive indicator of peripheral airways constriction in asthmatics (6) during exercise induced bronchospasm.

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Data analysis: Baseline data were first compared on the two test days, using the paired t-test. Subjects can properly serve as their own controls only if baseline values do not differ on the sham and acid exposure days. This comparison, made for each functional measurement, provides an estimate of intra-subject variation on separate days.

Results were then examined for overall changes associated with acid exposure. For each parameter, data were pooled into non-exposed (baselines and sham) and acid exposed groups. The means for each group were subjected to the unpaired t-test. Further, since we obtained several near-simultaneous measures of lung function, some of which might be correlated (for example, increases in R_{rs} are expected to correlate with decreases in V_{max50}) we tested all possible combinations of two or more variables measured nearly simultaneously for improved discrimination betweeen the two groups.

Finally, a three factor analysis of variance was carried out on each parameter. The factors were: exposure mode (M), either sham or acid; exercise (E), either absent or present just before measurement; a variable (C), reflecting measurement during baseline, first exposure, or second exposure. A response to acid exposure would appear as a significant interaction between M and C: the time course of measurements would differ on the acid day, compared to the sham day. Further, if incomplete neutralization (and hence airway irritation) occurred only on exposure during exercise, the functional measurements would produce an interaction between M and E: the effect of exercise would differ on the acid day, compared to the sham day. In this way the effect of time during the day, exercise and baseline changes between days may be accounted for separately, and distinguished from the influence of acid exposure.

RESULTS

Baseline data: The mean baseline measurements for the ten subjects appear in Table 4.1. There were no significant differences between the two test days for any functional parameter, although mean V_{max50} decreased by 11% and mean V_{isoV} rose by 55%. All other measurements varied by less than 10%. From this it appeared that the subjects would serve as their own controls, but changes in V_{max50} and V_{isoV} would have to be interpreted carefully in light of their baseline shifts.

Exhaled NH_3: Ammonia concentrations (Table 4.2) were in close agreement with those reported earlier (13,10). On both days NH_3 was higher after exposure, but not significantly, and there was no apparent influence of H_2SO_4 exposure. The rise may have been due to increased salivation stimulated by the mouthpiece.

Overall effect of exposure: Comparison of the non-exposed and exposed data group results (Table 4.3) indicated no significant difference for any single variable. The largest absolute value of Student's t resulted from DD_{50} ($.10>p>.05$) which decreased by 7% on exposure to H_2SO_4. No combination of parameters provided better discrimination between the two groups. Volume at isoflow increased by 19% during acid exposure, but the variance of this measure was large, and a random effect cannot be rejected.

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Table 4.1. Baseline data (mean ±s.e., n=10)

	Sham	H_2SO_4	paired t
R_{RS} cm H_2O/l/sec	2.83 ±0.47	2.70 ±0.41	0.48
$V_{max_{50}}$ l/sec	3.8 ±0.5	3.4 ±0.4	1.90
$V_{max_{75}}$ l/sec	1.7 ±0.4	1.6 ±0.3	0.84
N_2 slope $\%N_2$/l	.138 ±.012	.135 ±.014	0.25
r^2N_2*	.980 ±.004	.977 ±.003	0.64
DD_{50}	1.5 ±.03	1.5 ±0.1	-0.76
DD_{75}	1.4 ±.03	1.3 ±.03	1.04
V_{isoV} %VC	6.5 ±0.8	10.1 ±1.7	-1.36

*coefficient of regression of log($\%N_2$) on cumulative expired volume.

Table 4.2. Exhaled ammonia, $\mu g/m^3$

	Sham		H_2SO_4	
	pre exp	post exp	pre exp	post exp
mean ±s.e.	347±78	462±77	308±46	399±55
range	168-644	147-707	203-483	140-532
N	6	6	6	6

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Analysis of variance: The interaction of exposure mode with measurement period (Table 4.4, M x C) reached the significance criterion only for DD_{75}. On sham exposure DD_{75} decreased by 8%, while on acid exposure it increased by 4%, compared to the respective baselines (Figure 4.1). For several other variables there was a more impressive effect due to the interaction of exercise and measurement period. These effects are shown in Figures 2 and 3 for R_{rs} and V_{max50}, respectively. In the former, R_{rs} was consistently elevated after exercise, relative to the preceding resting value. This exercise effect decreased, however, with each succeeding challenge. A similar, but inverse, pattern was followed by V_{max50}. The pattern was not affected by the presence or absence of acid aerosol, as there was no mode-exercise interaction (Table 4.4). The consistency of the exercise-related changes in lung function was notable, compared to the barely-detected shifts in density dependence associated with H_2SO_4 exposure.

DISCUSSION

Despite our efforts to overwhelm the anticipated acid-neutralizing capacity of the mouth, there was little in our results to suggest that airway irritation occurred. There was a very small but consistent alteration of density dependence of maximum flow on acid exposure. The decrease in DD_{75} suggests a relative increase in the contribution of small airways to airflow resistance at flow limitation, which could reflect irritation and constriction striction of such airways. During sham exposure DD_{50} rose, suggesting a relative increase in more central airways' contribution to resistance at flow limitation. This sham response is consistent with central bronchoconstriction, possibly arising from exercise (see below). The calculated equilibrium diameter of the acid particles corresponds to a most likely deposition site in the lung periphery (14), reinforcing the suggestion of slight irritation in small airways. Although statistically significant, however, the mechanical changes associated with H_2SO_4 were very mild, especially when compared to the separate influence of exercise.

The finding of minimal mechanical response to H_2SO_4 is consistent with other recent reports. On exposure to similar particle sizes, and concentrations ranging from 100 to 1000 ug/m^3 (4,15) normal subjects displayed no changes in lung mechanics. Newhouse et al.(15), however, also measured the rate of clearance of radiolabeled particles from the tracheobronchial tree. Significant acceleration of clearance was found after exposure to concentrations of 1000 g/m^3, which suggests that central airway irritation did occur at these levels, but it was not reflected in changes in mechanical function. None of these investigators employed gases of altered density in their measurements.

While the apparent irritant effect of H_2SO_4 was minimal, analysis of variance indicated that after exercise our subjects consistently showed evidence of central airways constriction. The pattern of change in R_{rs} and V_{max} parameters after exercise and rest periods is similar to that seen in exercise-induced bronchospasm described in certain asthmatics (9). Others have shown that inhaling air at less than body temperature and saturation

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Table 4.3. Overall comparison of unexposed and exposed measurements (mean ±s.e.)

	Unexposed (n=78)	Exposed (n=40)	t	P
R_{RS} cm H_2O/l/sec	2.71±0.15	2.81±0.16	0.40	
$V_{max,50}$ l/sec	3.6±0.2	3.4±0.2	0.91	
$V_{max,75}$ l/sec	1.7±0.1	1.6±0.2	0.40	
DD_{50}	1.5±.02	1.4±.01	1.77	<.10
DD_{785}	1.4±.02	1.4±.02	0.59	
V_{isoV} %VC	8.4±0.6	10.0±0.4	0.81	

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Table 4.4. Analysis of variance: interactions of mode and time (M x C) and of mode and exercise (M x E)

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	M x C		M x E	
	F	P	F	P
R_{RS} cm H_2O/l/sec	0.2	<.90	0.3	<.90
$V_{max,50}$ l/sec	0.4	<.90	0.3	<.90
$V_{max,75}$ l/sec	0.5	<.50	0.3	<.90
N_2 slope %N_2/l	0.1	<.90		
r^2N_2*	1.7	<.25		
DD_{50}	2.5	<.25		
DD_{75}	5.1	=.05		
V_{isoV} %VC	0	<.99		

*coefficient of regression of log(%N_2) on cumulative expired volume

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Figure 4.1. Density dependence of maximum expiratory flow at 75% expired vital capacity (DD_{75}), derived from flow-volume maneuvers breathing air and a He-O_2 mixture (20 vol% O_2). Values of unity indicate no density dependence. Dashed line: Sham exposure; solid line: H_2SO_4 exposure. Vertical lines indicate standard error of mean (N=10).

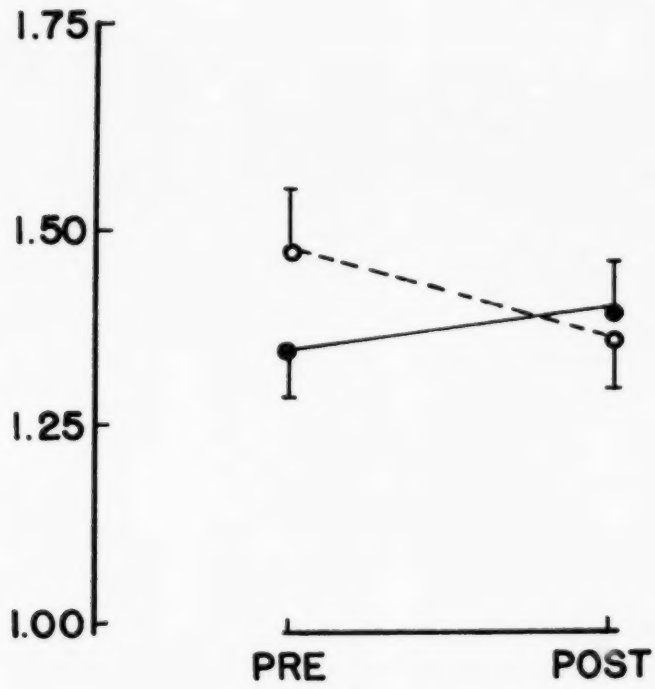

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Figure 4.2. Total respiratory flow resistance at 3 Hz (R_{rs}) prior to and during exposure. Ex: exercise sessions; dashed curve: sham exposure; solid curve: H_2SO_4 exposure. Vertical lines indicate one standard error of mean (N=10).

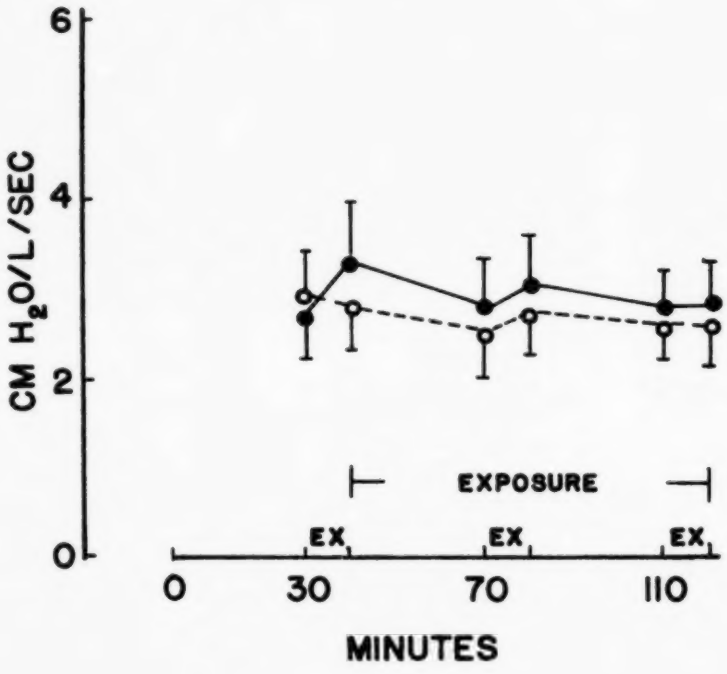

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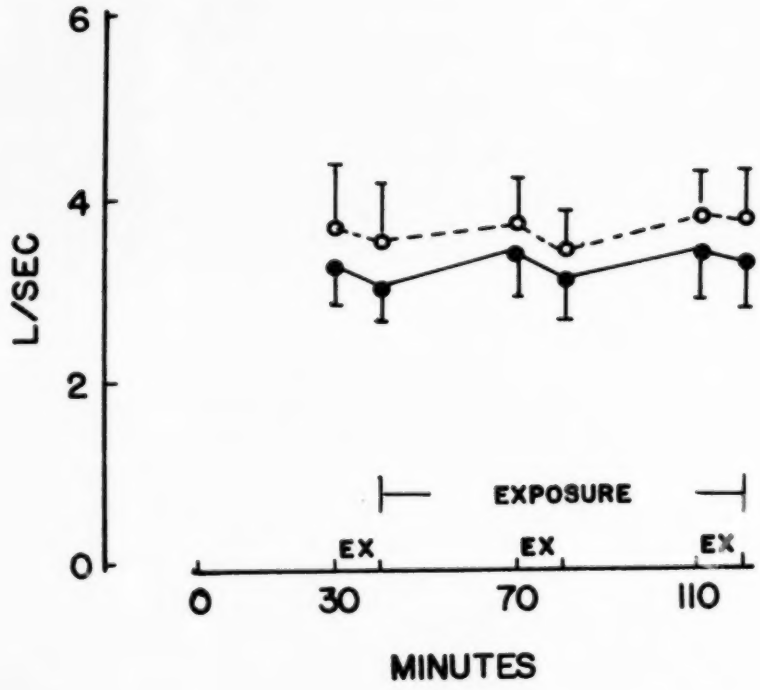
Figure 4.3. Maximum expiratory flow at 50% expired vital capacity (V_{max50}) plotted as in figure 2. (N=10).



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can trigger this response in asthmatics (8); a similar, but milder reaction to the same conditions may have occurred in our "normal" subjects. The subjects were not screened for the presence of atopy, and a fraction of them may have been atopic without respiratory symptoms. It is estimated that the occurrence of atopy without asthma may be 30-40% in this age group (5).

CONCLUSION

The acute irritant potency of H_2SO_4 aerosol, in concentrations of 500 ug/m^3 or more, is virtually undetectable in normal adults by usual measures of lung mechanical function. A more site-specific test, density dependence of maximum flow, provided evidence of very mild small airways constriction, but the work of others shows that measurement of tracheobronchial clearance is a much more sensitive indicator of response to H_2SO_4. Mechanical changes were slight or absent even under conditions strongly unfavorable for neutralization of H_2SO_4 by endogenous NH_3. Thus the influence of NH_3 conversion on lung function, if any, must be assessed by a method which is more sensitive than the ones used here. Subjects with known airway hypersensitivity, such as asthmatics, may provide a better means for testing the hypothesis, as might the use of a functional test such as clearance rate.

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4.5 REFERENCES

1. Amdur, M.O., Silverman, L. and Drinker, P. (1952). Inhalation of sulfuric acid mist by human subjects. A.M.A. Arch. Ind. Hyg. Occup. Med. 6:305-313.

2. Amdur, M.O., Bayles, J., Ugro, V., and Underhill, D.W. (1978). Comparative irritant potency of sulfate salts. Environ. Res. 16:1-8.

3. Anfield, R.D. and Warner, C. (1977) A study of industrial mists containing sulfuric acid. Ann. Occup. Hyg. 11:185-194.

4. Avol, E.L., Jones, M.P., Bailey, R.M., Chang, N.M., Kleinman, M.T., Linn, W.S., Bell, K.A. and Hackney, J.D. (1979) Controlled exposures of human volunteers to sulfate aerosols. Health effects and aerosol characterization. Am. Rev. Resp. Dis. 120:319-328.

5. Barbee, R.A., Lebowitz, M.D., Thompson, H.C. and Burrows, B. (1976) Immediate skin-test reactivity in a general population sample. Ann. Intern. Med. 84:129-133.

6. Brown, N.E. McFadden, E.R. and Ingram, R.H. (1977) Airway responses to inhaled histamine in asymptomatic smokers and nonsmokers. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 42:508-513.

7. Charlson, R.J., Vanderpol, A.H., Covert, D.S. and Waggoner, A.P. (1974) $H_2SO_4/(NH_4)_2SO_4$ background aerosol: optical detection in the St. Louis region. Atmos. Environ. 8:1257-1267.

8. Deal, E.C., McFadden, E.R., Ingram, R.H., Strauss, R.H. and Jaeger, J.J. (1979) Role of respiratory heat exchange in production of exercise-induced asthma. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 46:467-475.

9. Edmunds, A.T., Tooley, M. and Godfrey, S. (1978) The refractory period after exercise-induced asthma: its duration and relation to the severity of exercise. Am. Rev. Resp. Dis. 117:247-254.

10. Hunt, R.D. and Williams, D.T. (1977) Spectroscopic measurement of ammonia in normal human breath. Am. Lab. 9:10-22.

11. Koenig, J.Q., Pierson, W.E. and Frank, R. (1980) Acute effects of inhaled SO_2 plus NaCl droplet aerosol on pulmonary function in asthmatic adolescents. Environ. Res. 22:145-153.

12. Larson, T.V., Covert, D.S., Frank, R. and Charlson, R.J. (1977) Ammonia in the human airways: neutralization of inspired acid sulfate aerosols. Science. 197:161-163.

13. Larson, T.V., Covert, D.S. and Frank, R. (1979) A method for continuous measurement of ammonia in respiratory airways. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 46:603-607.

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14. Lippmann, M. (1977) Regional deposition of particles in the human respiratory tract. {In} "Handbook of Physiology. Reactions to environmental agents" (D.H.K. Lee, Ed.) Section 9, pp. 213-232.

15. Newhouse, M.T., Dolovitch, M., Obminski, G. and Wolff, R.K. (1978) Effect of TLV levels of SO_2 and H_2SO_4 on bronchial clearance in exercising man. Arch. Environ. Health. 33:24-32.

16. Perry, R.H., Ed. (1973) Chemical Engineers' Handbook, Fifth Edition. New York: McGraw-Hill, p. 3-64.

17. Sackner, M.A., Ford, D. Fernandez, R., Cipley, J., Perez, D., Kwoka, M., Reinhart, M., Michaelson, E.D., Schreck, R. and Wanner, A. (1978). Effects of sulfuric acid aerosol on cardiopulmonary function of dogs, sheep and humans. Am. Rev. Resp. Dis. 118:497-510.

18. Stanescu, D.C., Paltijn, J., Clement, J. and Van de Woestijne, K.P. (1972) Glottis opening and airway resistance. J. Appl. Physiol. 34:460-466.

19. Stevens, R.K. and Dzubay, T.G. (1978). Sampling and analysis of atmospheric sulfates and related species. Atmos. Env. 12:55-68.

20. Willeke, K. and Whitby, K.T. (1975) Atmospheric aerosols: size distribution interpretation. J. Air Poll. Control Assoc. 25:529-534.

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